

# Supplementary material

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1. Phylogenetic analysis
2. Bioinformatic analysis
3. Supplementary phylogenetic trees
4. Identification of poorly conserved homologues of NAD 2 and NAD 4L
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7. Table 1: Genes encoding mitochondrial proteins of *N. ovalis*

## 1. Phylogenetic analysis

Alignments of the representative protein sequences were constructed using ClustalW<sup>1</sup> and Muscle<sup>2</sup>. Unequivocally aligned positions were selected either with Gblocks<sup>3</sup>, or the most reliably aligned regions were selected manually with the help of Seaview<sup>4</sup>. Phylogenies were subsequently derived using two methods 1) Maximum Likelihood (ML) as implemented in MrBayes<sup>5</sup> using four gamma-distributed rate categories plus invariant positions and the Poisson amino acid similarity matrix, and 2) neighbour joining (NJ)<sup>6</sup> as implemented in ClustalW<sup>1</sup>, correcting for multiple substitutions using the Gonnet amino acids identity matrix<sup>7</sup>, and bootstrapping from 100 samples. The topologies shown in the figure correspond to the ML approach, and the values on the nodes indicate the posterior probability for the partition (ML) and its bootstrap value (NJ) respectively. Bootstrap values have been only indicated for the partitions that were retrieved by both methods and that were higher than 50%.

## 2. Bioinformatic analysis

Open reading frames with a lower size limit of 100 nucleotides were identified with ORF Finder (<http://www.ncbi.nlm.nih.gov/gorf/gorf.html>).

tRNAs were identified with tRNAscan-SE

(<http://www.genetics.wustl.edu/eddy/tRNAscan-SE/>)<sup>8</sup>

Potential mitochondrial import signals were detected with the network service MITOP (<http://mips.gsf.de/cgi-bin/proj/medgen/mitofilter>)<sup>9</sup>

Sequence similarity searches of deduced amino acid sequences were performed with BLASTX (<http://www.ncbi.nlm.nih.gov/BLAST/>)<sup>10</sup>

The nucleotide sequence similarity searches were conducted with BLASTN and FASTA

For the identification of potential mitochondrial genes a reciprocal Smith-Waterman sequence comparison was performed between the six-frames translated gDNA library of *N. ovalis* and the yeast and human mitochondrial proteins. Significant (e-value = 0.01) best bi-directional hits between both sets were selected.

1. Jeanmougin, F., Thompson, J.D., Gouy, M., Higgins, D.G. & Gibson, T.J. Multiple sequence alignment with Clustal X. *Trends Biochem. Sci.* **23**, 403-405 (1998).
2. Edgar, R.C. MUSCLE: multiple sequence alignment with high throughput and high accuracy. *Nucleic Acids Res.* **32**, 1792-1797 (2004).
3. Castresana, J. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol Biol Evol* **17**, 540-552 (2000).
4. Galtier, N., Gouy, M. & Gautier, C. SeaView and Phylo\_win, two graphic tools for sequence alignment and molecular phylogeny. *Comput. Applic. Biosci.* **12**, 543-548 (1996).
5. Huelsenbeck, J.P. & Ronquist, F. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**, 754-755 (2001).
6. Saitou, N. & Nei, M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**, 406-425 (1987).

7. Gonnet G.H., Cohen M.A. & Benner S.A. Exhaustive matching of the entire protein sequence database. *Science* **256**(5062), 1443-1445 (1992).
8. Lowe, T.M. & Eddy, S.R. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucl. Acids Res.* **25**, 955-964 (1997).
9. M.G. Claros & Vincens, P. Computational method to predict mitochondrially imported proteins and their targeting sequences. *Eur. J. Biochem.* **241**, 779-786 (1996).
10. Gish, W. & States, D.J. Identification of protein coding regions by database similarity search. *Nature Genet.* **3**, 266-272 (1993).

### Supplementary phylogenetic trees

The phylogenies were derived using MrBayes and neighbour joining (only Figs 6-10): the topologies correspond to the ML (MrBayes) approach, and the values at the nodes indicate the posterior probability for the partition and its bootstrap value, respectively.

Only values, which are higher than 50% are indicated.

H: encoded by the hydrogenosomal genome

N: nuclear-encoded

Only novel accession numbers are indicated in the legends (except those of *N. ovalis*, strain *Blaberus* spec. Amsterdam, which are displayed in Table 1)

Fig. S1: Nad5 of mitochondrial complex I (H)

Fig. S2: RPL2, mitochondrial ribosomal protein L2 (H)

Fig. S3: RPL14, mitochondrial ribosomal protein L14 (H)

Fig. S4: RPL 20, mitochondrial ribosomal protein L20 (N)

Fig. S5: 75 kD subunit of mitochondrial complex I (N)

Fig. S6: PDH E1a, pyruvate dehydrogenase subunit E1 alpha (N)

Fig. S7: PDH E1b, pyruvate dehydrogenase subunit E1 beta (N)

Fig. S8: PDH E2, pyruvate dehydrogenase subunit E2 (N)

Fig. S9: SDHa, succinate dehydrogenase subunit A (Fp) of mitochondrial complex II (N)

Fig. S10: SDHb, succinate dehydrogenase subunit B (Ip) of mitochondrial complex II (N)

Fig. S11: 24 kD subunit of mitochondrial complex I "24 kD" module of the hydrogenase (N)

Fig. S12: 51 kD subunit of mitochondrial complex I "51 kD" module of the hydrogenase (N)

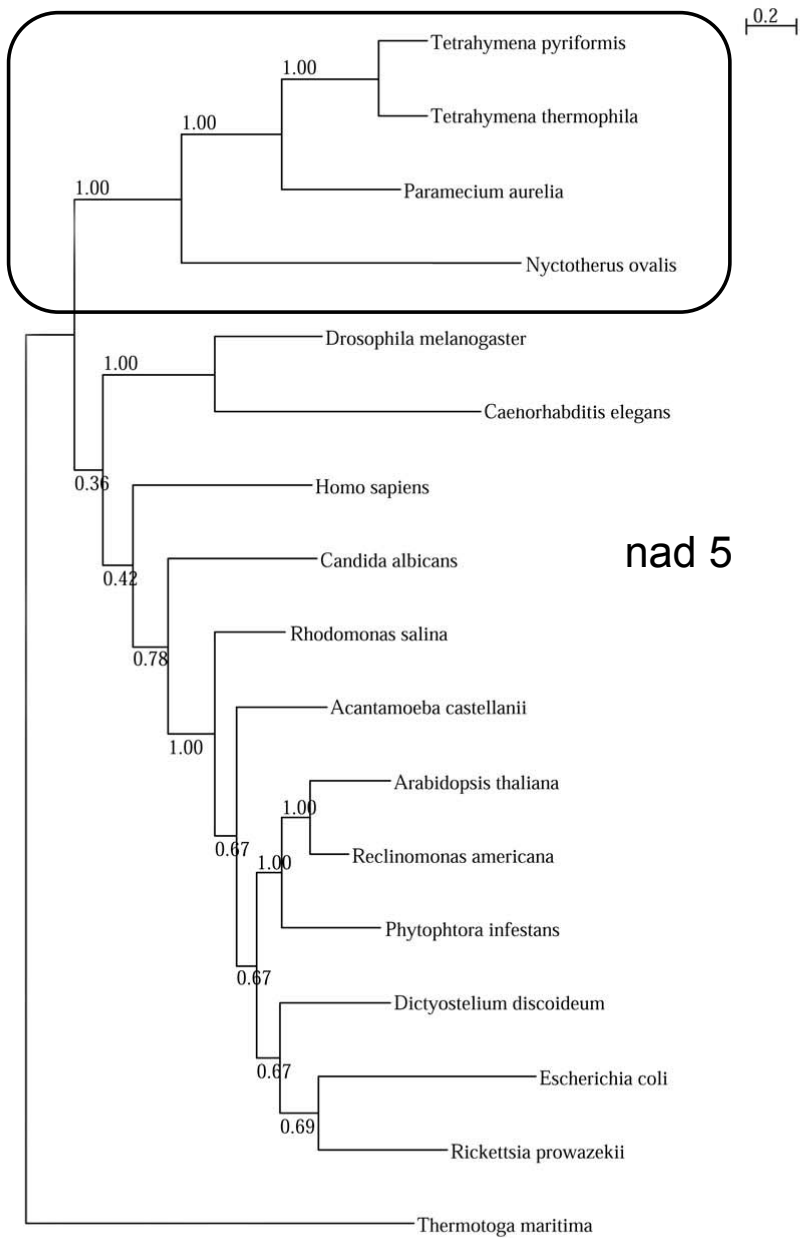


Fig. S1: Nad5 of mitochondrial complex I (H). Box: ciliate sequences

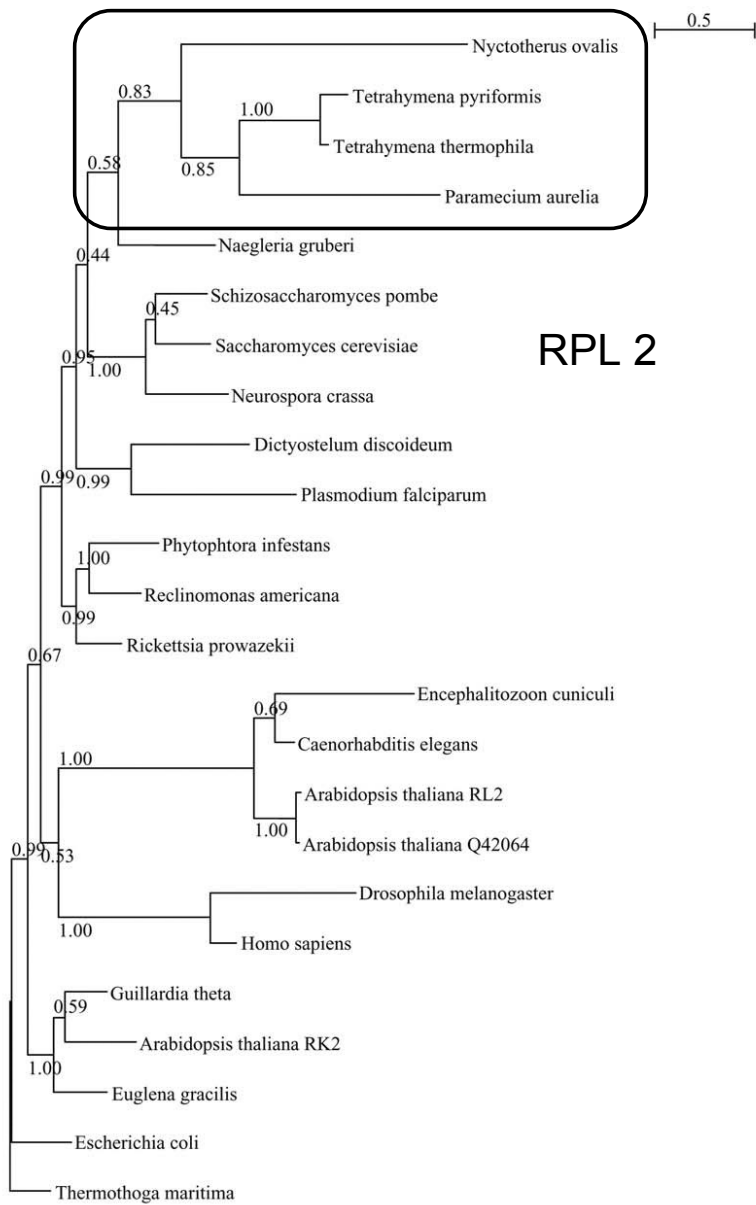


Fig. S2: RPL2, mitochondrial ribosomal protein L2 (H). Box: ciliate sequences

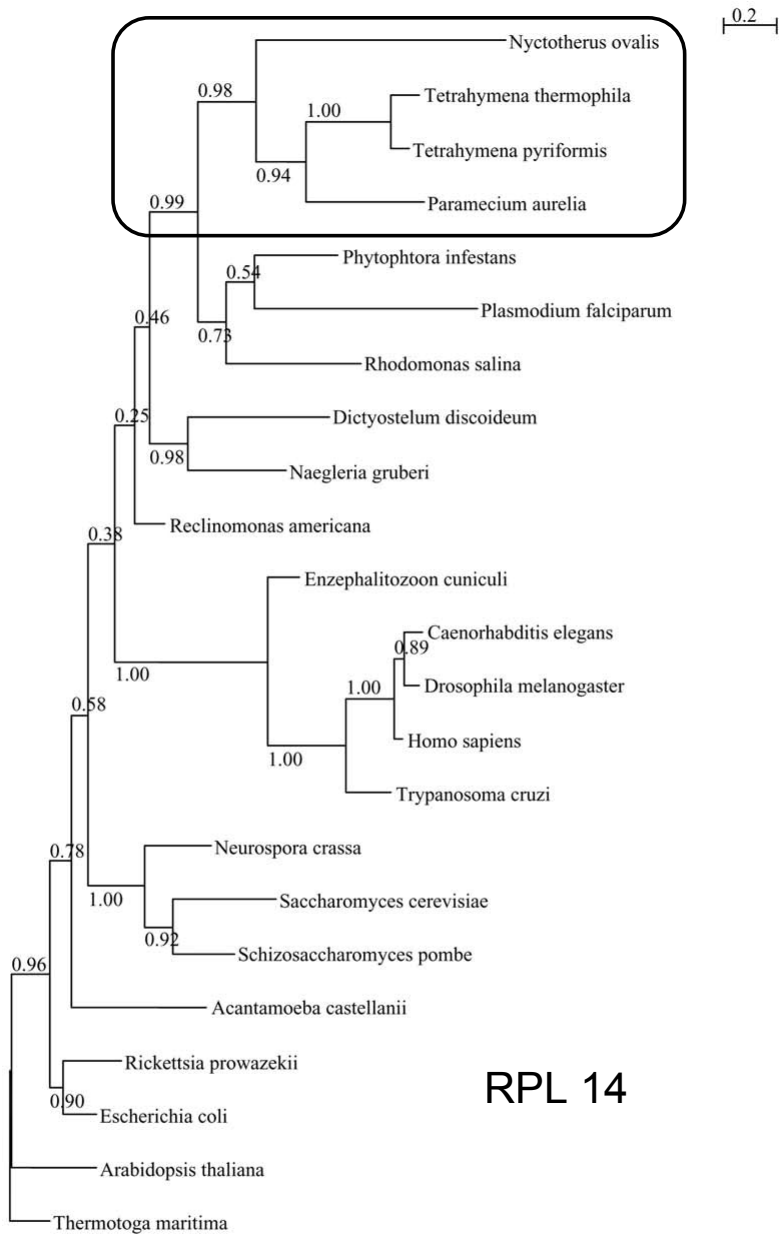


Fig. S3: RPL14, mitochondrial ribosomal protein L14 (H). Box: ciliate sequences

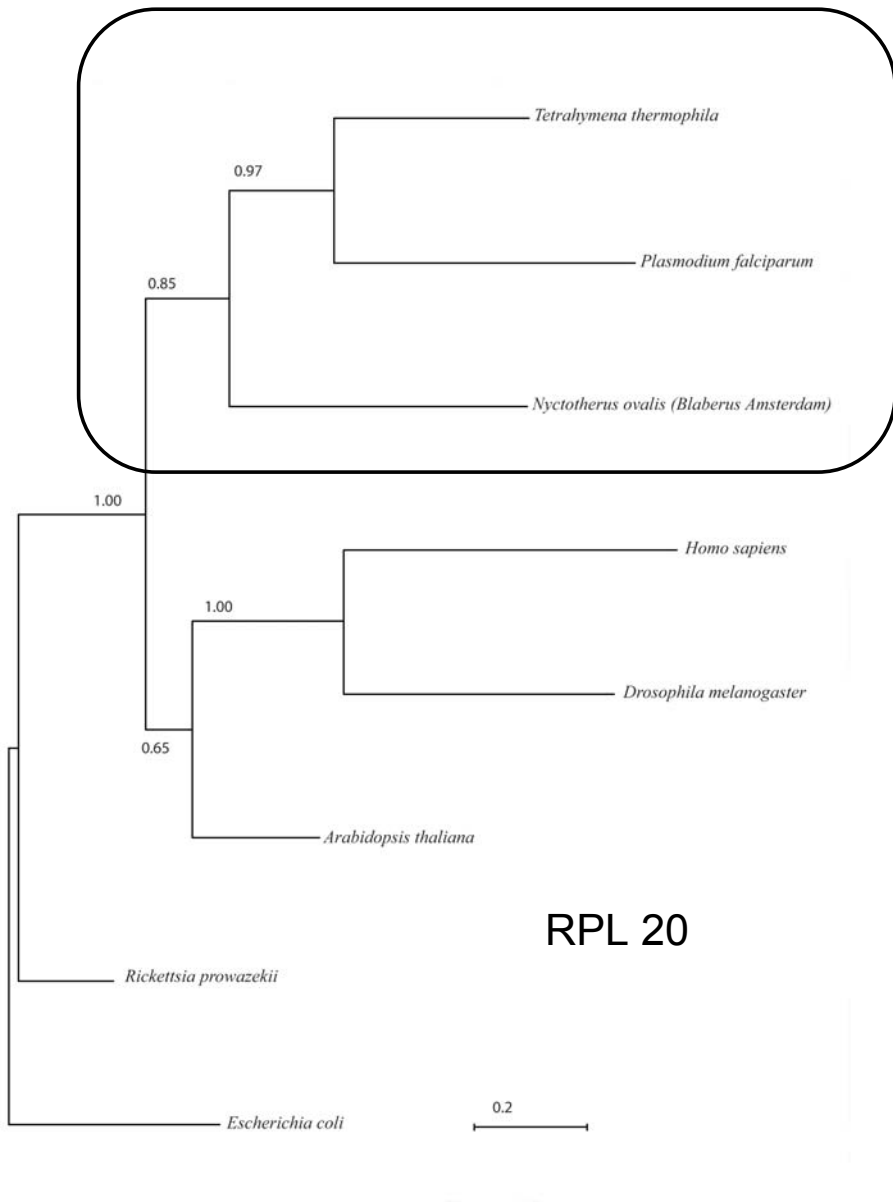


Fig. S4: RPL 20, mitochondrial ribosomal protein L20 (N).  
 Box: Alveolates (Ciliates plus Apicomplexans)

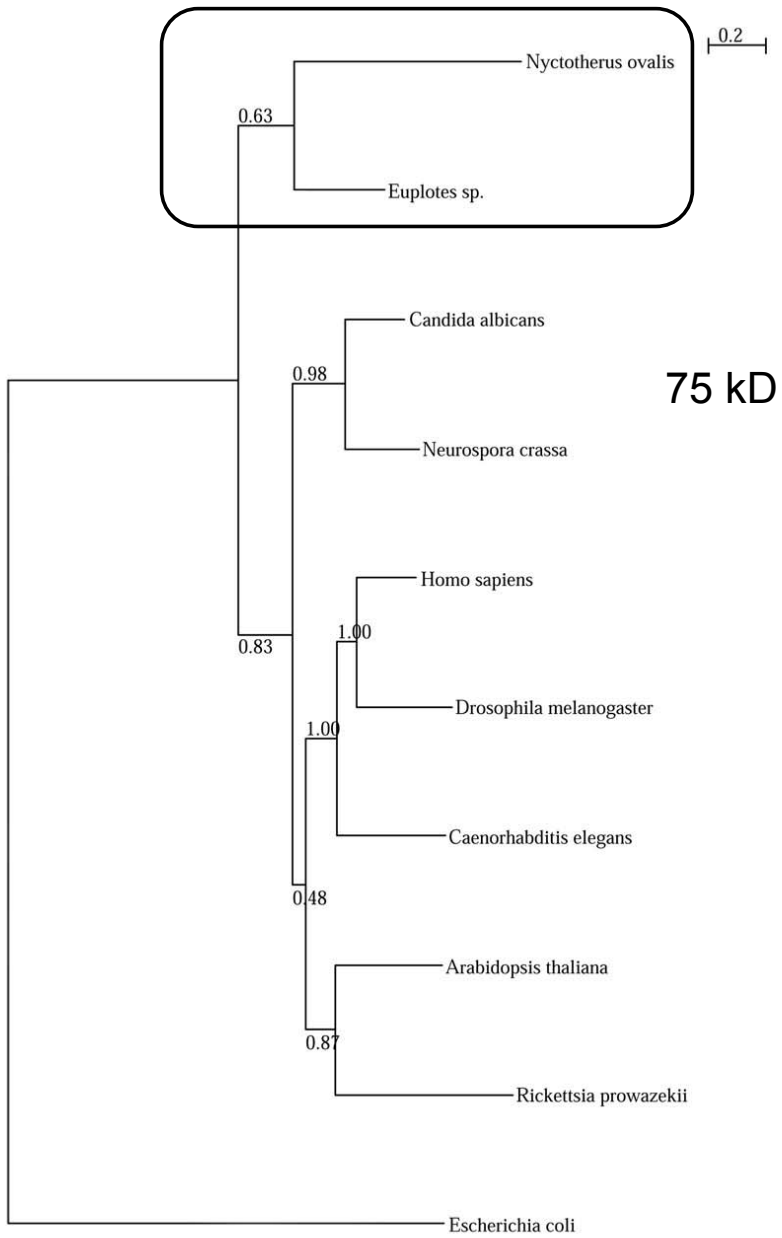


Fig. S5: 75 kD subunit of mitochondrial complex I (N). Box: ciliate sequences, Acc.nr. *Euplotes sp.* (\*\*\*\*\*)



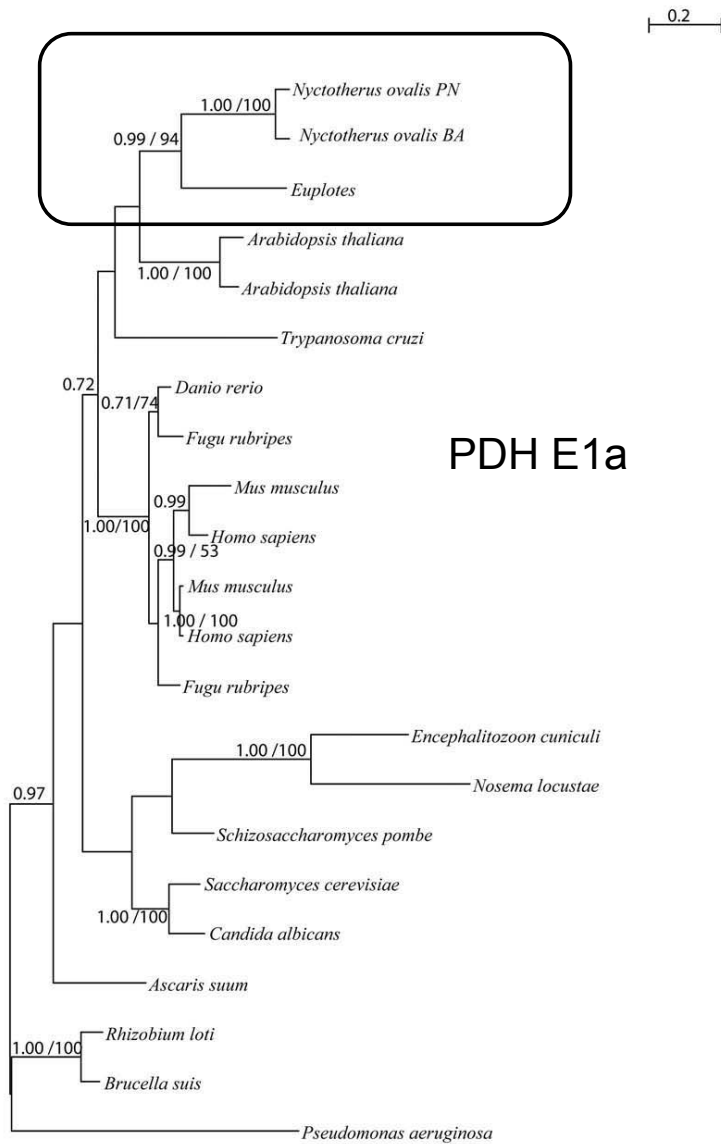


Fig. S6: PDH E1a, pyruvate dehydrogenase subunit E1 alpha (N).

Box: ciliate sequences,

*Nyctotherus ovalis PN*: strain *Periplaneta americana* Nijmegen (AY623923)

*Euplotes*: *Euplotes spec.* (AY623916)

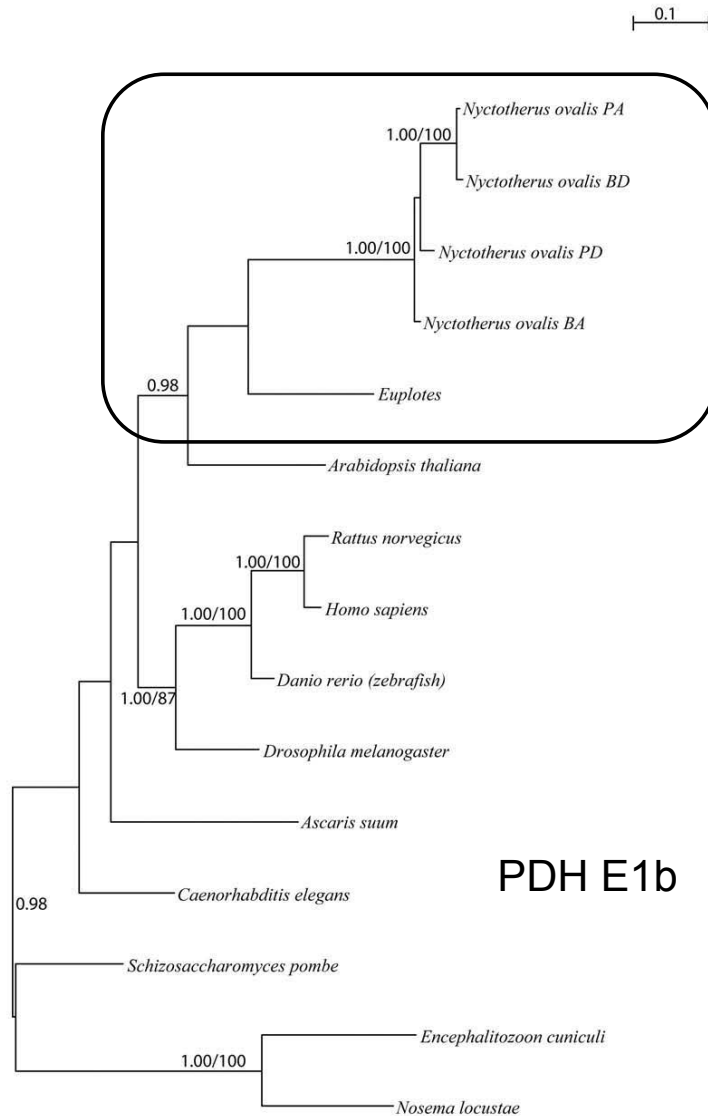


Fig. S7: PDH E1b, pyruvate dehydrogenase subunit E1 beta (N)

Box: ciliate sequences,

*Nyctotherus ovalis* BD, strain *Blaberus* spec. Düsseldorf (AY628685)

*Nyctotherus ovalis* PA, strain *Periplaneta americana* Amsterdam (AY628686)

*Nyctotherus ovalis* PD, strain *Periplaneta americana* Dar-es-salaam (AY628687)

*Euplotes* sp.(AY628682)

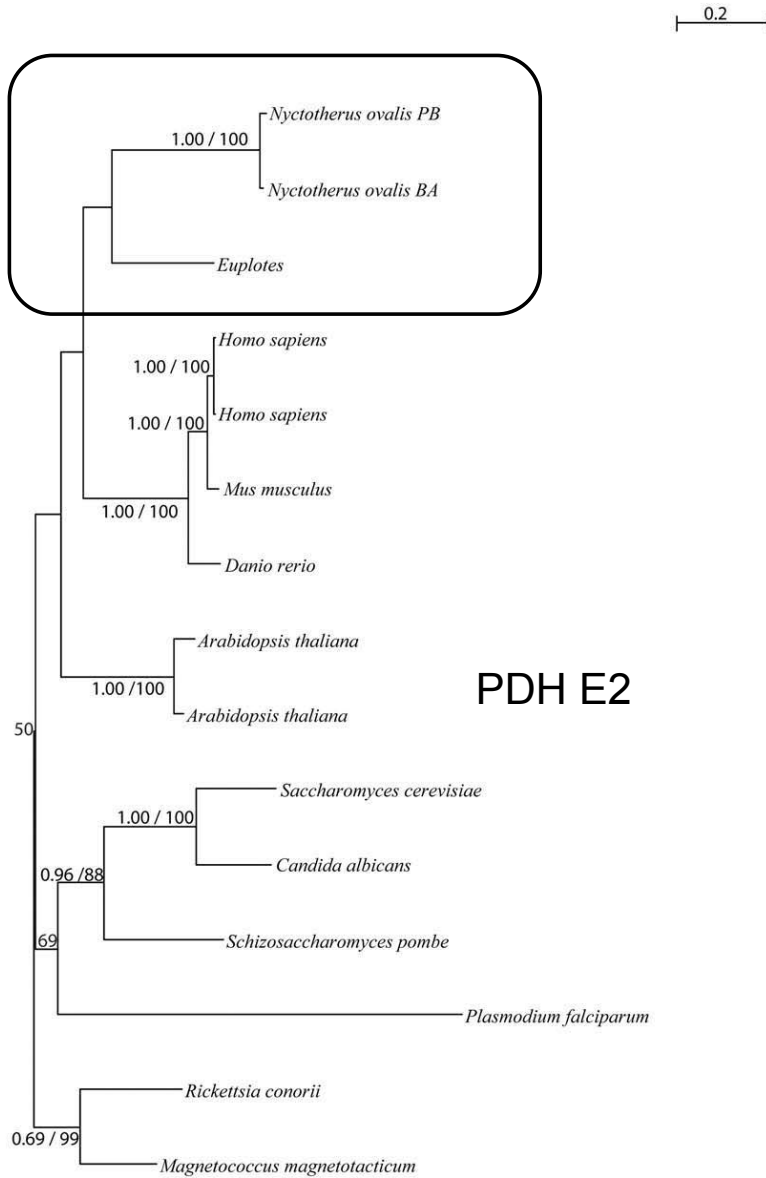


Fig. S8: PDH E2, pyruvate dehydrogenase subunit E2 (N),  
 Box: ciliate sequences,  
*Nyctotherus ovalis PB*, strain *Periplaneta americana* Bayer (AY623927)  
*Euplotes sp.* (AY623924)

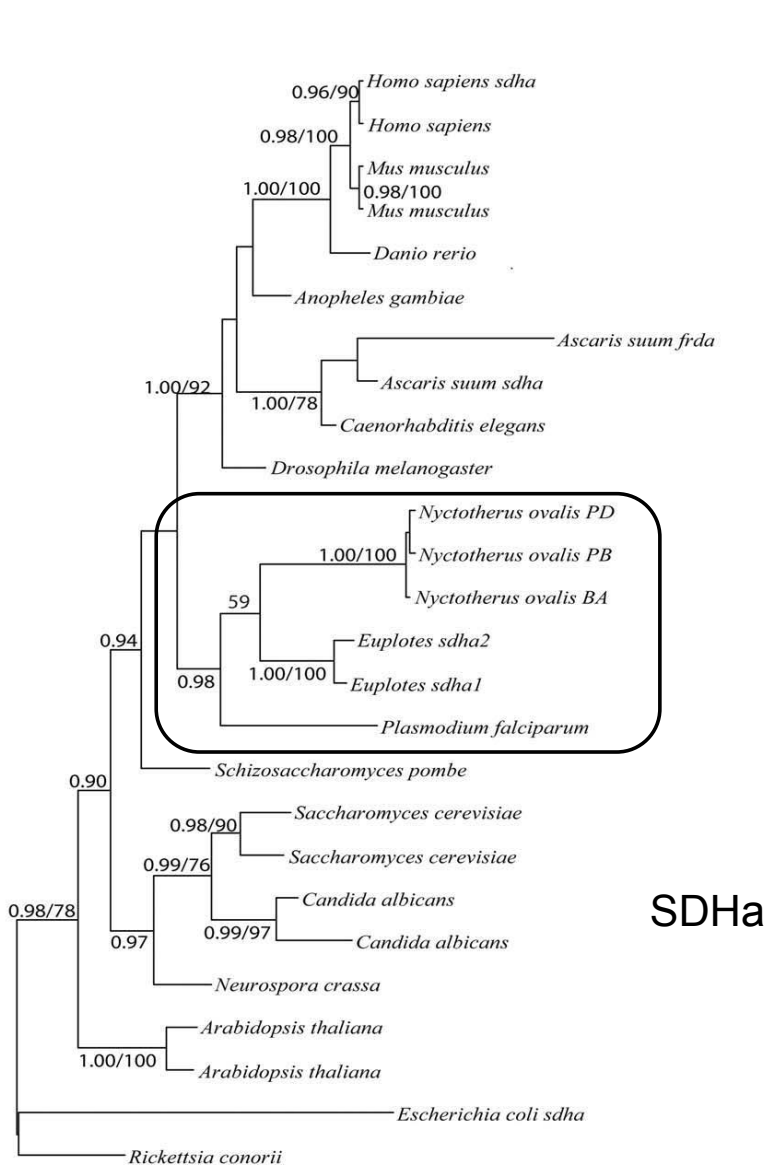


Fig. S9: SDHa, succinate dehydrogenase subunit A (Fp) of mitochondrial complex II (N)  
 Box: Alveolates (Ciliates plus Apicomplexans)  
*Nyctotherus ovalis PB*, strain *Periplaneta americana* Bayer (AY616153)  
*Nyctotherus ovalis PA*, strain *Periplaneta americana* Amsterdam (AY616154)  
*Nyctotherus ovalis BD*, strain *Blaberus* spec. Düsseldorf (AY616155)  
*Nyctotherus ovalis PD* strain *Periplaneta americana* Dar-es-salaam (AY616156)  
*Euplotes* sp. SDHa1 (AY616157)  
*Euplotes* sp. SDHa2 (AY616158)

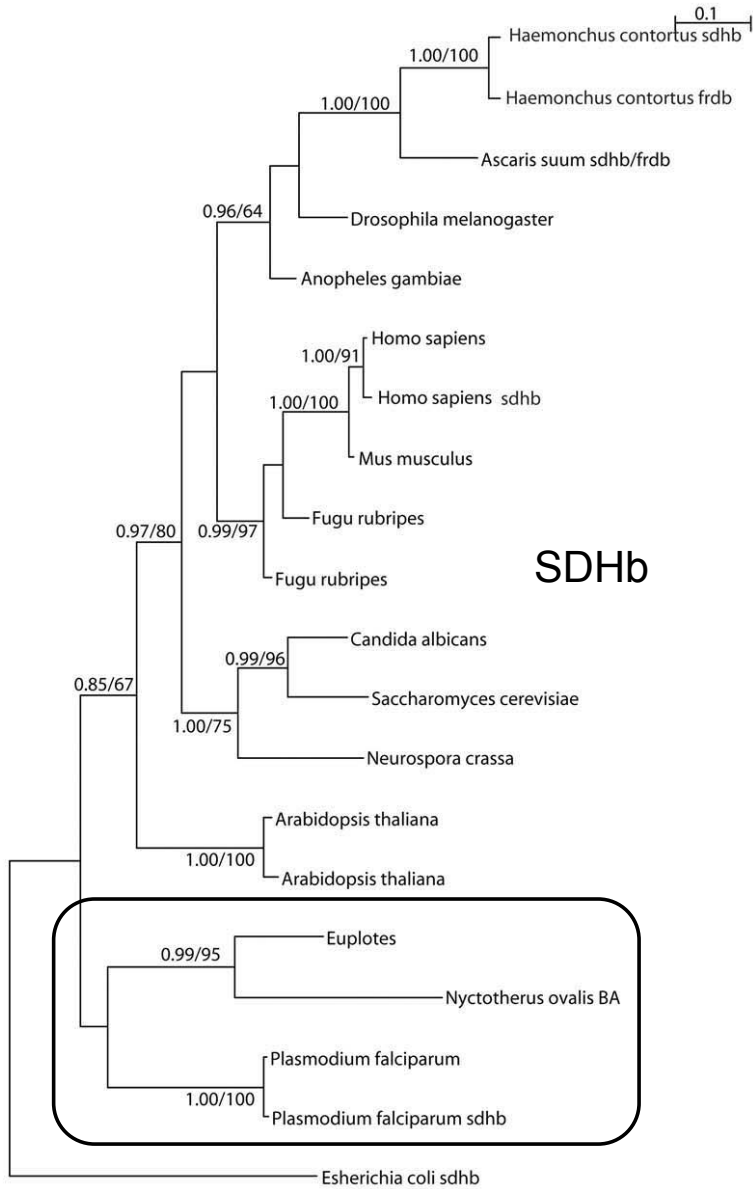


Fig. S10: SDHb, succinate dehydrogenase subunit B (Ip) of mitochondrial complex II (N)  
 Box: Alveolates (Ciliates plus Apicomplexans)  
*Euplotes* sp.(AY619984)

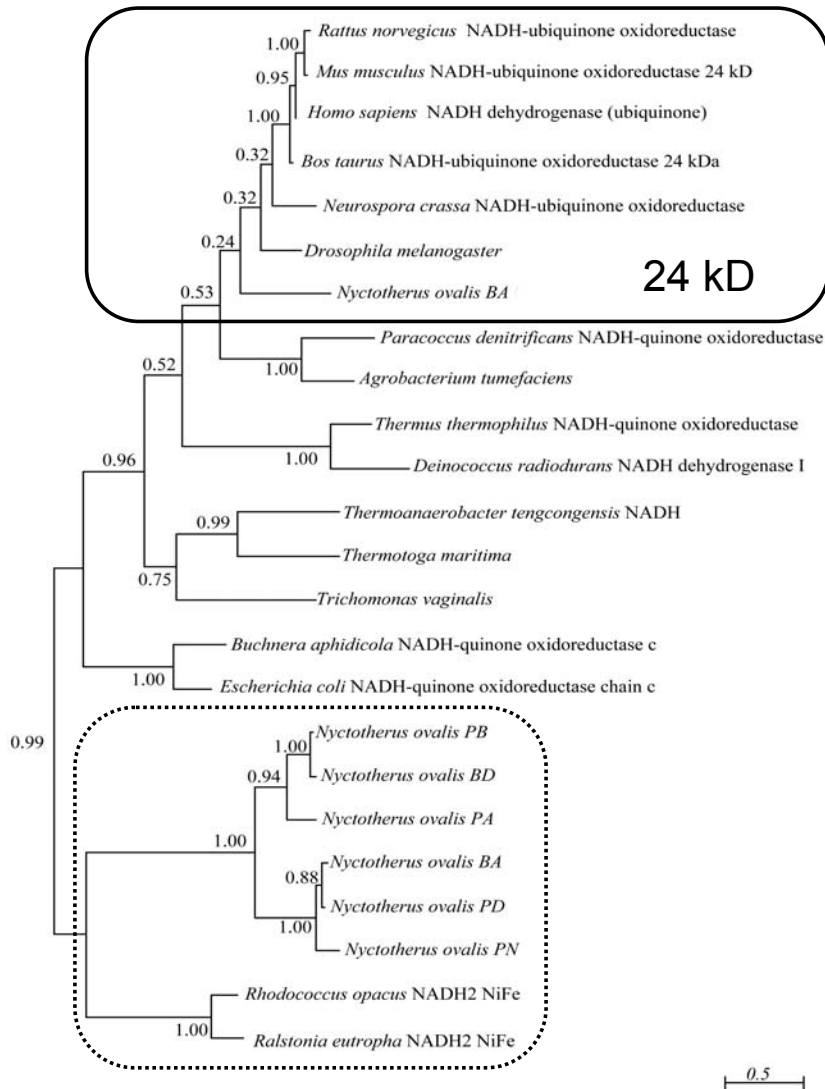


Fig. S11: 24 kD subunit of mitochondrial complex I/"24 kD" module of the hydrogenase (N)  
 Box with solid line: 24 kD subunits of mitochondrial complex I  
 Box with dotted line: hydrogenase modules  
*Nyctotherus ovalis BD*, strain *Blaberus* spec. Düsseldorf (AY608628)  
*Nyctotherus ovalis PA*, strain *Periplaneta americana* Amsterdam (AY608629)  
*Nyctotherus ovalis PB*, strain *Periplaneta americana* Bayer (AY608630)  
*Nyctotherus ovalis PD*, strain *Periplaneta americana* Dar-es-salaam (AY608631)  
*Nyctotherus ovalis PN*, strain *Periplaneta americana* Nijmegen

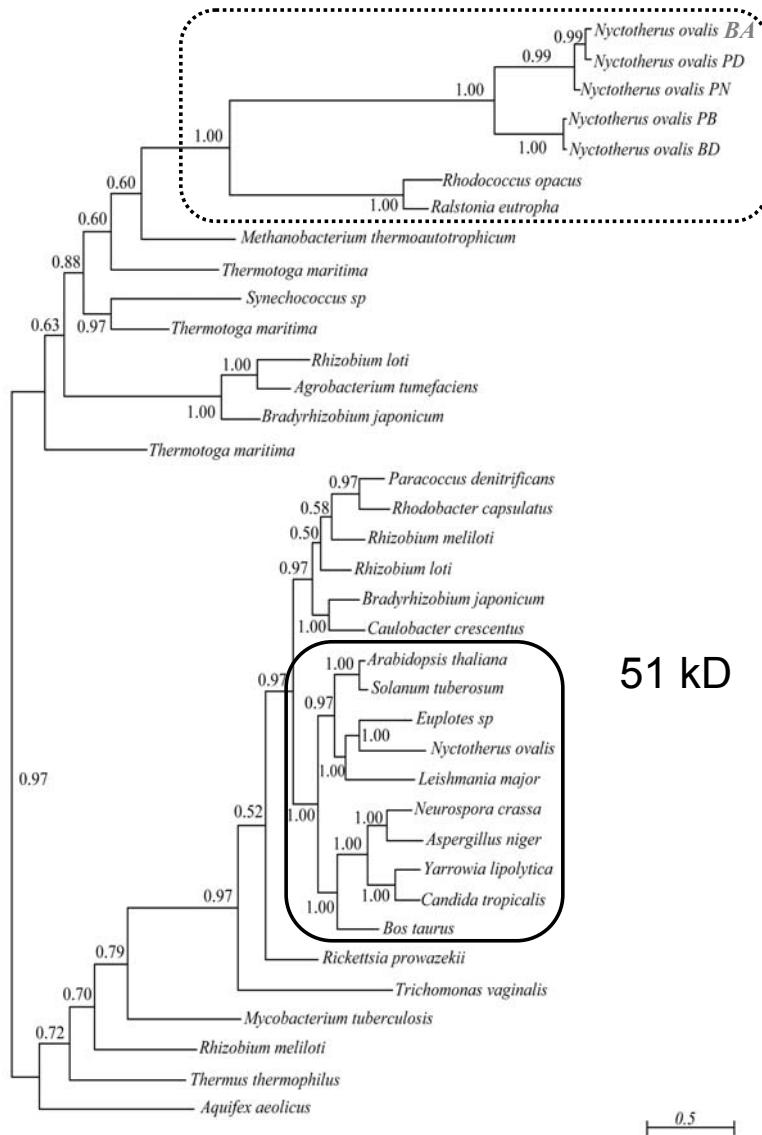


Fig. S12: 51 kD subunit of mitochondrial complex I "51 kD" module of the hydrogenase (N)  
 Box with solid line: 51 kD subunits of mitochondrial complex I  
 Box with dotted line: hydrogenase modules  
*Nyctotherus ovalis* PA, strain *Periplaneta americana* Amsterdam (AY608635)  
*Euplotes* sp. (AY608636)  
*Nyctotherus ovalis* BD, strain *Blaberus* spec. Düsseldorf (AY608628)  
*Nyctotherus ovalis* PB, strain *Periplaneta americana* Bayer (AY608630)  
*Nyctotherus ovalis* PD, strain *Periplaneta americana* Dar-es-salaam (AY608631)  
*Nyctotherus ovalis* PN, strain *Periplaneta americana* Nijmegen

#### **4. Identification of poorly conserved homologues of NAD 2 and NAD 4L**

The organelle encoded ORFs potentially encoding NAD 2 and NAD 4L, have been analysed according to Brunk, C. F., Lee, L. C., Tran, A. B. & Li, J. Complete sequence of the mitochondrial genome of *Tetrahymena thermophila* and comparative methods for identifying highly divergent genes. *Nucleic Acids Res.* **31**, 1673-1682 (2003).

For NAD 2, four TM domains have been identified, i.e. *T. pyriformis* 20-42, 62-82, 99-116, 137-159; *T. thermophila* 15-37, 58-80, 95-114, 135-157; *N. ovalis* 15-37, 59-60, 95-117, 140-162. For NAD 4L, there are 3 TM domains, i.e. *T. pyriformis* 4-21, 26-48, 58-80; *T. thermophila* 4-21, 26-48, 58-80; *N. ovalis* 2-19, 26-48, 58-80. These data clearly support our interpretation.

For the alignments see the following pages.



# Fig. S13: CLUSTAL X (1.4b) MULTIPLE SEQUENCE ALIGNMENT

File: /mnt/cammsa20/comics/jagabald/HYDROGENOME/FIRST/aln2.ps

Date: Fri Oct 8 17:21:54 2004

Page 1 of 2

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idopsi MKAEFVRIILPHMFLAVSPEIFIINATSILLIHGVVFTSKKYDYPPLASNVGWLGLLSVLITLILLAAAGAPLLITIAHLFWNNLFRDRNFTYFCQIFLLISTAGTISMCFDSSDQERFDAFEFIVLPLPTRGMLFMISAHDLIAMYLA
.pyrif
a_ther
ettisia -----MLLLLPEITLT-----LIALLGQCFALMIPNKNRIIYNIVILLCIISIFLTFKYSSY-----EGIWHSFATERNIGISKSIIILLFTIVSLIIYRDYSILVGETLKFEFITLMLLSIVGIFVAITSSRNFLLLFCG
.aurel
E_coli -----MDVTPLMRVDGFAMILYTGLVLLASLATCTFAYPWLEGYNDNKDEFYLLVLLIAALGGILLANATTWRSFLFG
.sapie
8_NAD2 -----MNPAAQPVLYSTIFAGTLITALSSHWFFTWVG
ruler 1.....10.....20.....30.....40.....50.....60.....70.....80.....90.....100.....110.....120.....130.....140.....150.
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mad2_N AIEFQSLCLYVLAAFKRNSLFSVEAGLYKVFVLAGAFSSGIMLFGISILYGFVGTGFEDLGLKLLIFSSNESLSSSGIILGIVFISVGLLFKIYAVPFHVWVDPVYQGSPTIVTAFFAIVPSISVLTLLTRLYSSVLHDLIQYWQPVFIFCS
idopsi AIEFQSLCFYVIAASKRKSEFSTEAGSKYLILGAFSSGILLFGCSMIYGSTGATHFDQLAKILTGYEITGARSS-GIFMGILSIAVGLFLKITA VPFHMWAPDIYEGSPTPVTAFLSIAPKISISANILR--VSIYGSYGATLQQIFFFCS
.pyrif
a_ther
ettisia CMELTALTSYALAGFKLNDIKSSEGALKYFILGSLVSCSLFGISIFTYFGGSIQFDDILHQLN--NDSEIKR-GLIIGIVLFLSSIFPKLASSPLHFVIPDVYEGSPISSVITYFTAASKIGMVIVLLNISKLLIIGNYYPINYNLIKIIA
.aurel
E_coli GIELISLPLFGLVGYAFRQKRSLEASIKYITLSAAASSFLFGMALVYAOAGDLSFVALGKNLG---DGMLNDALLAGFGLMIVGLGFKLSLVPFHLWTPDVYQGAPAPVSTFLATASKIATFGVVMRLFLYAPVGDSEQVRRGAGDYR
.sapie
8_NAD2 GLEMNMLAFIPVLTKKLNP-RSTEAAIKYFLTQATASMILLMAILFNNMMSGQWAMTNTTINYS-----SLMIMMAMAM-----KLGMAFFHFVWPEVVTGTPLTSGLLLTWQKLAFISIMYQ-----ISPSSLNVSLLLTLS
ruler .....160.....170.....180.....190.....200.....210.....220.....230.....240.....250.....260.....270.....280.....290.....300.
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mad2_N SIASMLIGSLGALSQRRIKRLIAYSAGHVGYVLLIATSTGTP--EGIRGLLVYIVYIIMSACFFTILLSLRKRENTQIVMIDELRALYKSNPILSLTIAIVLFSMAGIPPLAGFFSKLYVFLPALHEGLFLLVVVVGIASVISAIVYYLQ
idopsi SIASMLIGALAAQAQTRVKRPLAHSSIGHVGYIRTFGFCGTI--EGIQSLLIGIFTYALMTDFAFIVSALR---QTRVKYLADLGA LAKTNPISAITFSITMFSYAGIPPLAGFCCKFYLFFAALGCGAYFLAPVGVVTSVIGRFYYLR
.pyrif -----MSVFNNVWINNDLNS-----YGLSILLNINILNYLIVFMLIISIILITDI--SKFKSLNQKFEFNSYN-FILYSLIFSLLSMAGIPPLGFTGKFLAAILFSVFKSQYILLIFMTIILNIFGMFYFIQ
a_ther -----SIFSNWINNDLNS-----YGLSILLNININILNYLIVFMLIISVILLTN--SKFKSLNQKFEFNSYN-FILYSLIFSLLSMAGIPPLGFTGKFLAAILYSSFKSQY-LLIFMTIILNIFGMFYFIQ
ettisia AILSMLFGAFGAIROTSLKRLMAYSTIINIGYVLTGVLLHNQ--EGYKAALLYILYAVVSI GFFTCLIMLFG--KDVNDNASFKTIEGTAETHKTAALISIVMFSMIGIPPLTGFFGKYLLFYQAINKKEFTIAYCGIFTSVVAIFYLLK
.aurel -----MRAHLHCELAFSFGKYFYSTSFLNLLMINLMFS-----KLGALFFLNALYLLAALFFFLEFNK---VALLKMSVQIYYFNIF-FFKFFVLIFFLNLAGIPPLGFFLFLFFLFFKTNLAFILIFLGFNMAITFFYLS
E_coli RFASITIFGNLMAISQTNIKRLLGYSSISHLGYLLVALIAIQTGEMSMEAVGVYLAGYLFSSIGAFGVVSLMSSPYRGPDADSLFSYRGLFWHRPILAAVMTVMMLSLAGIPMTLGFIGKFYVLAAGVQAHLWLVGAVVVGSAIGLYYYLR
.sapie SILSINMAGSWGGLNQTLRKILAYSSITHMGWMMAVLPY-----NPNMTIILNLTYYIILTTAFLLINLNS--S-TTTLLSRTWNKLT-WLTPLIPSTLLSLGGLPPLTGFLPKWAIIEEFTKNNSLIIPMTIATITLLNLYFYLR
8_NAD2 -----MHIYSMMSLLE-----QYVTLIALHVLGYVLAGTLLIYLLSQVI-----TTNTVFLNALGHDM-LIRTCLLICLSSMAGLPPVLGFWTKLYLLLVGSFNGNMTLIIIMSLMMLVGLIYYIV
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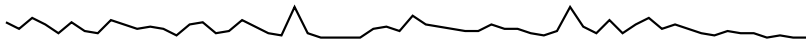
# CLUSTAL X (1.4b) MULTIPLE SEQUENCE ALIGNMENT

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Date: Fri Oct 8 17:21:54 2004

Page 2 of 2

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idopsi  RLVKRMFFDTPRTWILYEPMDRKNK----SLLLAMTSFFITSSLLYPSPLFSVTHQMALSSYL
.pyrif  QNLRFVVKKNKSSILNYKNYYVNIYAI SLNIVLINFNFNFFGILFSDFIILNYISSFLFI
a_ther  QNLRFVVKKNKSSILNYKNYYVNIYSITLNIILLNFFNFFG-IFLSDLIILNYISSYIYI
ettsia  KVVKAMYFSKK---IAIIKLPMQY-----GLLLINYLVLGFLFSGFIILF-----
.aurel  STVKSFVNRKQASVLNSFNFFIRA----ELSFLYFNFFYFFLFFAFFFLDSTFLIFLNLF
E_coli  RVAVSLYLHAFEPGRDAPSNWQYSAG-GIVVLI SALLVLVLGVWFQPLISIVRLAMPLM--
_sapie  RLIYSTSITLLF-MSNNVKKMKWQFEHTKPTPFLPTLIALTLLLPISPFMLMIL-----
8_NAD2  VIASYTITEVPHG-LELLTLNGNT----CSELMSTCCMLLLGSIFLQDTYMFLYNMVL----
ruler  .....460.....470.....480.....490.....500.....510..
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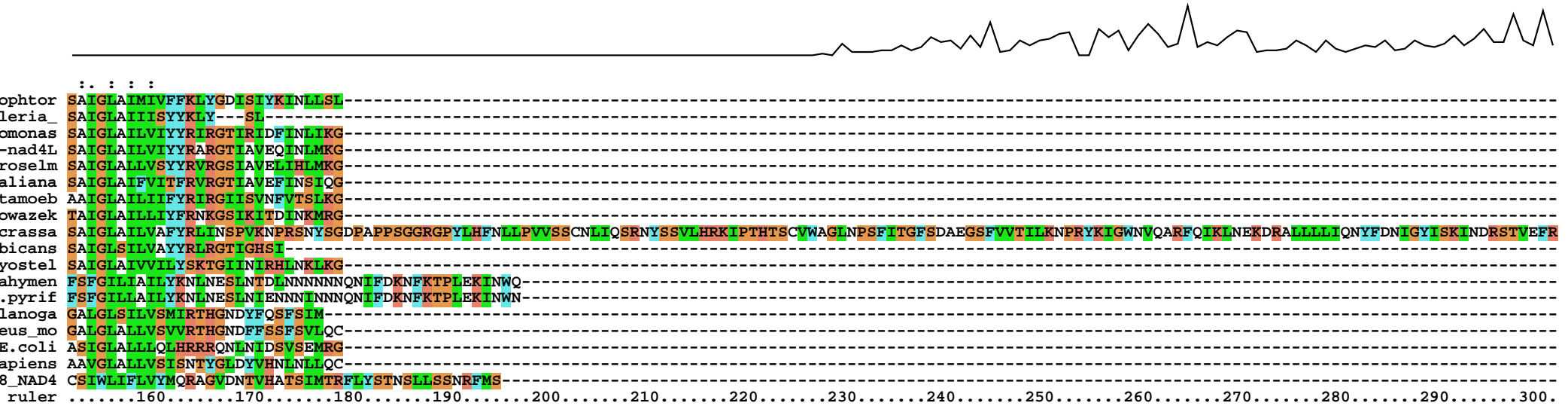
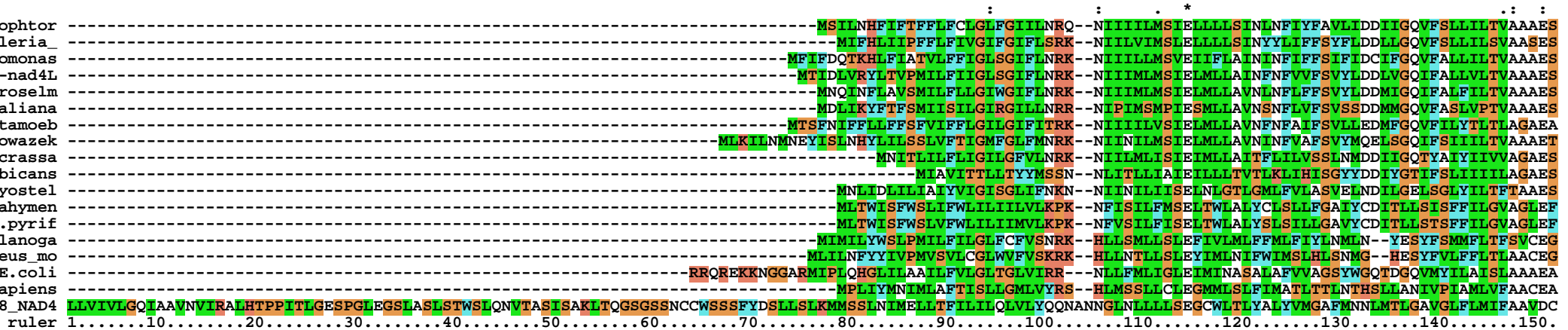


# Fig. S14: CLUSTAL X (1.4b) MULTIPLE SEQUENCE ALIGNMENT

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Date: Tue Oct 12 18:17:17 2004

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# CLUSTAL X (1.4b) MULTIPLE SEQUENCE ALIGNMENT

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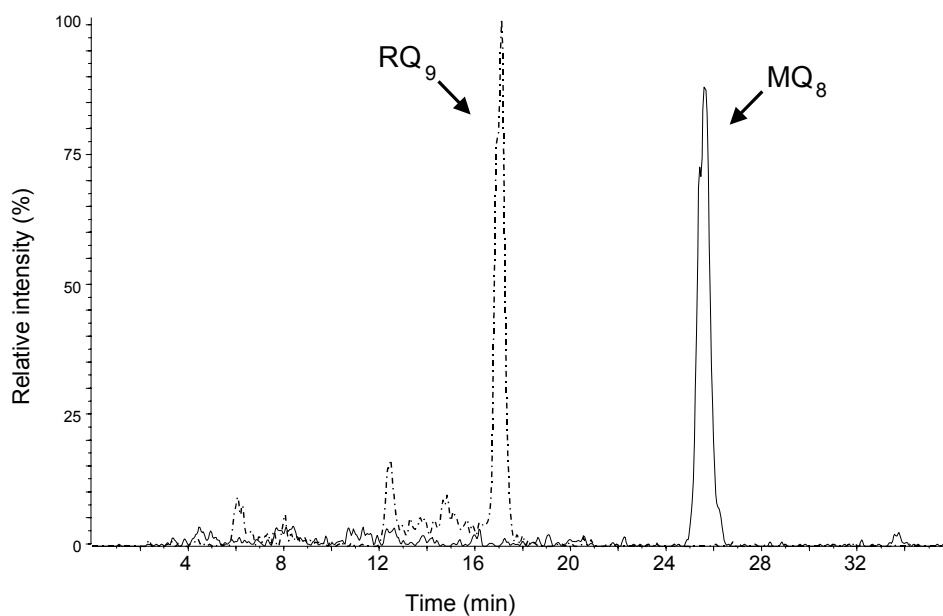
Page 2 of 2

```
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leria_ -----
omonas -----
-nad4L -----
roselm -----
aliana -----
tamoeb -----
owazek -----
crassa RVSDITSLNNLIIPHFKEYQLINKYGDLVIFKQIVSLMLENKHTTLEGLKELLEHRASLNWGLSKTLKESFPSIIPVKRVKIENNLLSNLSSLPPLPRGGNWVAGFSSGEANFFITMSGTKVWLRFSIAQDSRDILLKSLVKFFNCYI
bicans -----
yostel -----
ahymen -----
.pyrif -----
lanoga -----
eus_mo -----
E.coli -----
apiens -----
8_NAD4 -----
ruler .....310.....320.....330.....340.....350.....360.....370.....380.....390.....400.....410.....420.....430.....440.....450.
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ophtor -----
leria_ -----
omonas -----
-nad4L -----
roselm -----
aliana -----
tamoeb -----
owazek -----
crassa LAQYKNRNVCEFIVTKINDIITYIIPFFDQYKLEGSKYNDYVKFKEAALIKNKEHLTEKGLNKITTELNKSLPPPASLEGGMNKNI
bicans -----
yostel -----
ahymen -----
.pyrif -----
lanoga -----
eus_mo -----
E.coli -----
apiens -----
8_NAD4 -----
ruler .....460.....470.....480.....490.....500.....510.....520.....530.....
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## 5. Supplementary data quinone determination, Fig. S15



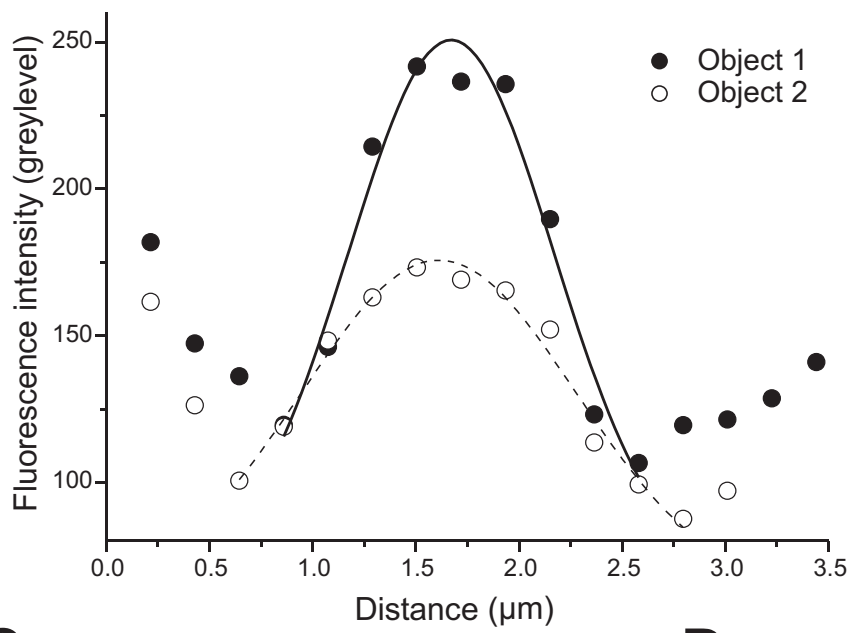
Quinones were separated by liquid chromatography on a Lichrospher RP-18e column and detected by a Sciex API 300 triple quadrupole mass spectrometer with an atmospheric pressure chemical ionisation source as described before (Hoffmeister et al 2004). Quantification of the quinones was performed by selective reaction monitoring taking  $[M+H]^+$  as a precursor ion for ubiquinones (UQ<sub>6 to 10</sub>), rholoquinone (RQ<sub>6 to 10</sub>) and menaquinone (MQ<sub>6 to 8</sub>) and the quinone headgroup as specific product ion ( $m/z$  197.1, 182.1 and 187.1 for UQ, RQ and MQ, respectively). Calibration curves were prepared with quinone standards.

M. Hoffmeister, A. van der Klei, C. Rotte, K.W. A. van Grinsven, J. J. van Hellemond, K. Henze, A. G. M. Tielens and W. Martin, *Euglena gracilis* rholoquinone:ubiquinone ratio and mitochondrial proteome differ under aerobic and anaerobic conditions. *J. Biol. Chem.* 279 (2004) 22422-22429.

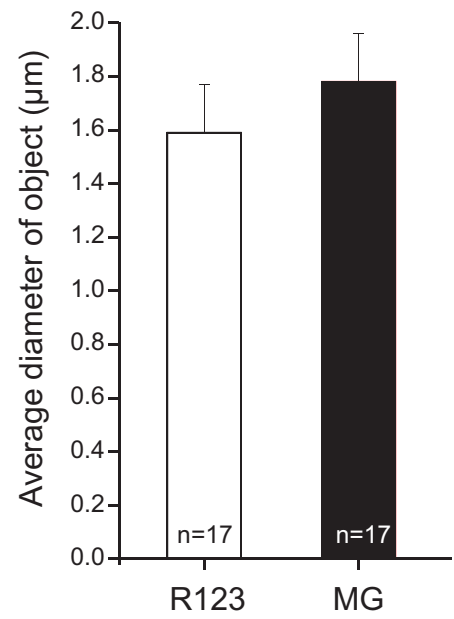
## 6. Light microscopy

**Visualization of hydrogenosomes in living *N. ovalis* cells using CLSM.** Mitochondria were stained with Mitotracker green FM<sup>®</sup> (MG; 10 min, 5  $\mu$ M) or Rhodamine-123 (R123; 3 min, 100  $\mu$ M; see Fig. 3 of main text). Rhodamine-123 accumulates in organelles with a highly negative membrane potential (Chen, L. B. *et al.* Probing mitochondria in living cells with Rhodamine 123. *Cold Spring Harbor Symp. Quant. Biol.* **46**, 141–155, 1982). Mitotracker green FM<sup>®</sup> stains the same organelles confirming the presence of a mitochondrial-type of membrane. To confirm the correspondence between the hydrogenosomes identified by electronmicroscopy and immunocytochemistry<sup>15</sup> and the “mitochondria” identified by CLSM, images were recorded on a Noran OZ<sup>®</sup> videorate confocal microscope, realtime averaged at a rate of 30 img/s using a NA 1.4 x60 objective and a confocal section thickness of 3  $\mu$ m (Koopman, W. J. H. *et al.*, Reference 28). Next, images were background corrected and contrast optimized to highlight R123 and MG-positive structures. Methanogenic endosymbionts are much smaller (Fig. 3 of manuscript; ref 19); they do not stain with R123 under these conditions (not shown). (A) The diameter of the fluorescent structures was determined using the width at half maximal amplitude of a Gaussian curve fitted to the intensity profile transecting the centre of each object. (B) This analysis revealed similar object diameters for R123 (AVG $\pm$ SE: 1.59 $\pm$ 0.18  $\mu$ m, n=17 mitochondria) and MG (1.78 $\pm$ 0.18  $\mu$ m, n=17), which matches the size of (fixed and dehydrated) hydrogenosomes identified in the electron micrographs. (C) The addition of DMSO (used to improve the solubility of Rotenone and Fenazaquine) does not hamper the staining of the hydrogenosomes by R123 even at concentrations of 10% v/v. (E) The R123 signal persisted for at least 4 hours after staining. (D) shows the effect of incubation with Fenazaquine (an inhibitor of mitochondrial complex I) on R123 staining, which is the same as Rotenone, Piericidin, and MPP<sup>+</sup>.

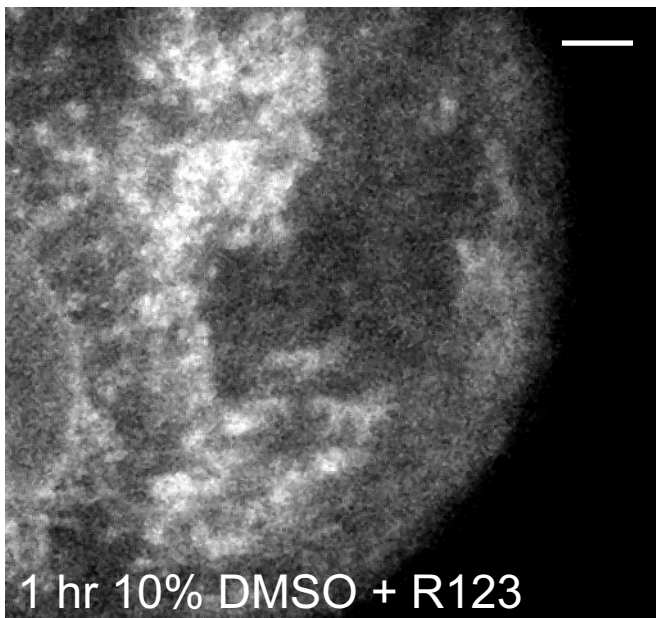
Fig. S16 A



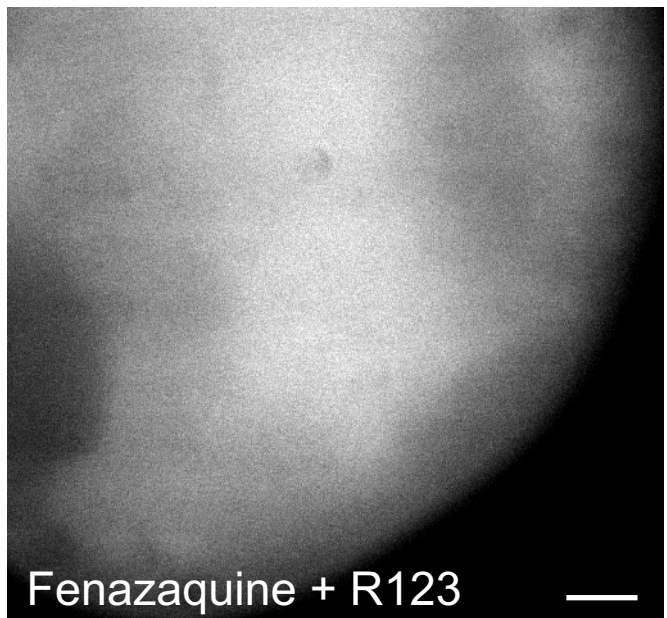
B



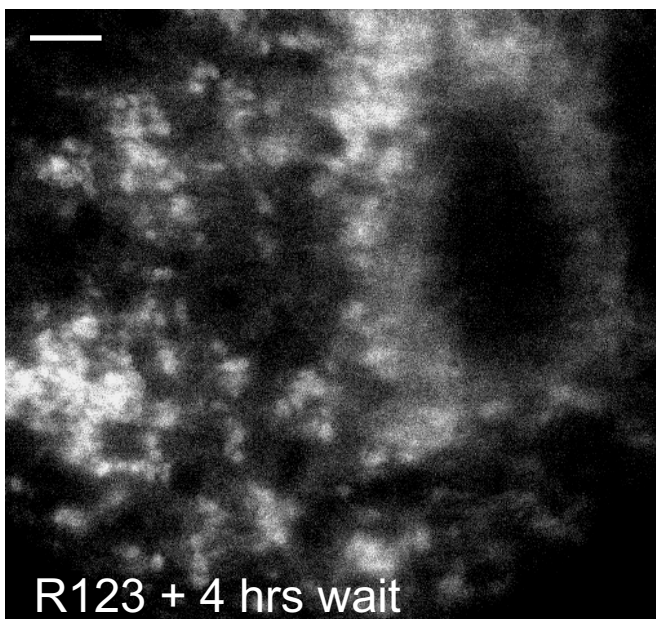
C



D



E



<b>Table S1: Genes encoding mitochondrial proteins</b>	<b>clone</b>	<b>match</b>	<b>e-value</b>	<b>Local</b>	<b>Codon use</b>	<b>cDNA</b>	<b>Target. signal</b>	<b>Accession number</b>
<b>mitochondrial complex I</b>								
NAD2	mtg-1	mt genome Tetrahymena	3.00E-04	H	Mt		no	AJ871267
NAD4 L	mtg-1	mt genome Tetrahymena	0.2	H	Mt		no	AJ871267
NAD5	mtg-1	mt genome Tetrahymena	8.00E-59	H	Mt	Y	no	AJ871267
NAD7	mtg-1	mt genome Tetrahymena	2.00E-90	H	Mt	Y	no	AJ871267
24kD		Hsa-IPI00018770.2	8.00E-54	N	Nuc		Y	AY628688
51kD		Hsa-IPI00028520.2	1.00E-178	N	Nuc	Y	Y	AY608632
75kD		Hsa-IPI00002871.1	1.00E-28	N	Nuc		Y	*****
<b>mitochondrial complex II</b>								
SDH a		YJL045W	0	N	Nuc	Y	Y	AY616152
SDH b		Hsa-IPI00011216.2	2.00E-80	N	Nuc	Y	Y	AY619980
<b>mt protein synthesis</b>								
Putative ribosomal RNA methyltransferase 2	HK530_1	Hsa-IPI00006026.1	3.00E-36	N	Nuc			AJ871313
<b>mt ribosomal proteins</b>								
RPL 2	mtg-1	mt genome Tetrahymena	4.00E-11	H	Mt			AJ871267
RPL 14	mtg-1	mt genome Tetrahymena	0.052	H	Mt			AJ871267
RPL 20	HK2972_2	Hsa-IPI00013706.1	1.00E-13	N	Nuc		?	AJ871314
<b>mt tRNA</b>								
tRNA tyrosine	mtg-1			H	rna			AJ871267
<b>mt rRNA</b>								
12S rDNA (SSU)	mtg-1			H	rna			AJ871267
<b>mt catabolism/energy metabolism</b>								
PDH E1a		Hsa-IPI00029237.1	1.00E-102	N	Nuc	Y	Y	AY623917
PDH E1b		Hsa-IPI00003925.1	1.00E-119	N	Nuc	Y	Y	AY628683
PDH E2		Hsa-IPI00021338.1	1.00E-105	N	Nuc	Y	Y	AY623925
[Fe] Hydrogenase				N	Nuc	Y	Y	AY608627
Acetyl-coenzyme A synthetase 2 (EC 6.2.1.1)	HK700_2	Sce-ACS2_YEAST	5.00E-34	N	Nuc		no	AJ871315
Adenylate kinase 2 (EC 2.7.4.3)	HK2418_2	Sce-KAD2_YEAST	2.00E-20	N	Nuc		no	AJ871316
adenylate kinase 2 isoform c =HK2418	HK2589_0	Hsa-IPI00172460.1	4.00E-19	N	Nuc			AJ871317
Probable D-lactate dehydrogenase [cytochrome] (EC 1.1.2.4)	HK865_2	Sce-DLD3_YEAST	3.00E-38	N	Nuc		Y	AJ871318
succinyl-CoA ligase	HK 088	Sce-SUCB_YEAST	5.00E-42	N	Nuc		Y	AJ871319
Succinyl-CoA:3-ketoacid-coenzyme A transferase, mitochondrial	HK1860_2	Hsa-IPI00026516.1	2.00E-52	N	Nuc		Y	AJ871320
Glycerol kinase	HK1998_0	Hsa-IPI00027424.1	7.00E-38	N	Nuc		C	AJ871321
<b>mt carrier family</b>								
AAC		Hsa-IPI00022891.1	1.00E-100	N	Nuc		no	AF480921
Putative mitochondrial carrier protein PET8 YNL003C_Chr	HK197_2	Sce-PET8_YEAST	0.001	N	Nuc		no	AJ871322
<b>mt import/processing</b>								
Mitochondrial processing peptidase alpha subunit	HK2671	Sce-MPPA_YEAST	2.00E-19	N	Nuc		?	AJ871323



HSP 60	HK1444			N	Nuc		Y	AJ871324
HSP 70	HK2256			N	Nuc		no	AJ871325
Heat shock protein HSP82 YMR186W_Chr	HK1228_1	Sce-HS82_YEAST	4.00E-77	N	Nuc		no	AJ871326
TOM 34	HK1184_2	Hsa-IPI00009946.2	1.00E-22	N	Nuc		no	AJ871327
Mitochondrial protein import protein MAS5 (Protein YDJ1)	HK087_0	Sce-MAS5_YEAST	2.00E-20	N	Nuc		C	AJ871328
Stress-70 protein, mitochondrial precursor	HK2165_0	Hsa-IPI00007765.2	2.00E-43	N	Nuc		C	AJ871329
<b>amino acid metabolism</b>								
Branched-chain amino acid aminotransferase, mitochondrial	HK118_0	Sce-BCA1_YEAST	8.00E-20	N	Nuc		no	AJ871330
Putative alanine aminotransferase, mitochondrial precursor	HK1384_1	Sce-ALAM_YEAST	3.00E-48	N	Nuc		C	AJ871331
Glutamate dehydrogenase 2, mitochondrial precursor	HK1446_1	Hsa-IPI00027146.1	6.00E-60	N	Nuc		?	AJ871332
Cystathionine beta-synthase (EC 4.2.1.22)	HK1495_2	Sce-CBS_YEAST	3.00E-41	N	Nuc		no	AJ871333
Serine hydroxymethyltransferase, mitochondrial precursor	HK1649_1	Hsa-IPI00002520.1	1.00E-74	N	Nuc		?	AJ871334
Saccharopepsin precursor (EC 3.4.23.25)	HK453_2	Sce-CARP_YEAST	0.007	N	Nuc		C	AJ871335
<b>fatty acid metabolism</b>								
Propionyl-CoA carboxylase alpha chain, mitochondrial precursor	HK1083	Hsa-IPI00007245.1	1.00E-68	N	Nuc		Y	AJ871336
Long-chain-fatty-acid--CoA ligase 1 (EC 6.2.1.3)	HK1758_1	Hsa-IPI00013161.1	8.00E-17	N	Nuc		?	AJ871337
<b>miscellaneous</b>								
Ubiquitin--protein ligase RSP5 (EC 6.3.2.-)	HK1235_0	Sce-RSP5_YEAST	3.00E-29	N	Nuc		C	AJ871338
Hypothetical 50.6 kDa protein in RPL14B-GPA1 intergenic	HK1300_0	Sce-YHG4_YEAST	2.00E-18	N	Nuc		C	AJ871339
P68-like protein YNL112W_Chr	HK1426_2	Sce-DBP2_YEAST	4.00E-16	N	Nuc		?	AJ871340
Probable alpha-glucosidase YJL216C (EC 3.2.1.20)	HK1471_0	Sce-MAXS_YEAST	2.00E-09	N	Nuc		no	AJ871341
Kinesin heavy chain	HK1504_2	Hsa-IPI00012837.1	1.00E-51	N	Nuc		Y	AJ871342
Dynamin-related protein DNM1 (EC 3.6.1.50)	HK1679_1	Sce-DNM1_YEAST	7.00E-04	N	Nuc		C	AJ871343
Protein phosphatase 2C gamma isoform	HK044_0	Hsa-IPI00006167.1	6.00E-26	N	Nuc		no	AJ871344
Protein-tyrosine phosphatase 1 (EC 3.1.3.48)	HK195_0	Sce-PTP1_YEAST	3.00E-06	N	Nuc		C	AJ871345
Mitogen-activated protein kinase FUS3 (EC 2.7.1.37)	HK025_1	Sce-FUS3_YEAST	8.00E-54	N	Nuc		no	AJ871346
Protein disulfide isomerase precursor	HK1749_2	Hsa-IPI00010796.1	2.00E-32	N	Nuc		no	AJ871347
Carbon catabolite derepressing protein kinase (EC 2.7.1.-)	HK1831_0	Sce-SNF1_YEAST	2.00E-67	N	Nuc		no	AJ871348
Peptidyl-prolyl cis-trans isomerase C, mitochondrial	HK2173_0	Sce-CYPC_YEAST	3.00E-20	N	Nuc		C	AJ871349
Chromosome XVI reading frame ORF YPL249C	HK2221_0	Sce-Q12344	0.009	N	Nuc		C	AJ871350
Adenylate cyclase (EC 4.6.1.1) (ATP pyrophosphate-lyase)	HK2231_0	Sce-CYAA_YEAST	1.00E-10	N	Nuc		no	AJ871351
Ras-related protein SEC4	HK2488_0	Sce-SEC4_YEAST	2.00E-34	N	Nuc		C	AJ871352
mouse MPV17 protein	HK2632_0	Sce-Q06563	2.00E-21	N	Nuc		no	AJ871353
Prohibitin	HK2676_2	Hsa-IPI00017334.1	2.00E-52	N	Nuc		Y	AJ871354
Hypothetical 34.9 kDa protein in SMI1-PHO81 intergenic	HK2676_2	Sce-YG4W_YEAST	2.00E-44	N	Nuc		Y	AJ871354
Ubiquitin YKR094C_Chr	HK2854_1	Sce-UBIQ_YEAST	2.00E-35	N	Nuc		C	AJ871355
Protein C21orf2	HK2943_2	Hsa-IPI00014472.	2.00E-32	N	Nuc		no	AJ871356
YME1 protein (EC 3.4.24.-) (TAT-binding homolog 11)	HK307_1	Sce-YME1_YEAST	1.00E-33	N	Nuc		C	AJ871357
Serine/threonine protein phosphatase 2A, catalytic subunit	HK316_1	Hsa-IPI00008380.1	7.00E-78	N	Nuc		no	AJ871358
GTP-binding protein YPT1 (Protein YP2)	HK473_1	Sce-YPT1_YEAST	2.00E-38	N	Nuc		no	AJ871359
ATP-dependent metalloprotease YME1L	HK900_1	Hsa-IPI00099529.1	9.00E-35	N	Nuc		C	AJ871360

Midasin (MIDAS-containing protein)	HK971_0	Sce-MDN1_YEAST	3.00E-14	N	Nuc		?	AJ871361
C: partial sequence (C-terminus), no N-terminus yet								
?: low probability, potential intron around start codon								
nucleus ; H: hydrogenosome; Y: yes; Nuc: nuclear; mt: mitochondrial								
mtg-1: 12 kb clone of hydrogenosomal genome								